Supplementary materials

Table S1. Questionnaire

1. What is your normal starting material? (Tick all that apply)

- Conditioned cell culture media (serum added)
- Conditioned cell culture media (serum-free)
- Blood plasma
- Blood serum
- Urine
- Cerebral spinal fluid
- Milk
- Other (please specify below)

2. What is your typical starting volume?

- < 1 mL
- 1 - 5 mL
- 5 - 20 mL
- 20 - 100 mL
- >100 mL
- Comments

3. What is your primary isolation technique?

- Ultracentrifugation
- Precipitation (please state method/manufacturer)
- Density gradient (please state method/manufacturer)
- Ultra-filtration (please state method/manufacturer)
- Magnetic bead capture (please state method/manufacturer)
- A combination of methods (Please state below)
- Other (please specify below)
- Comments

4. Which additional cleaning and/or purification technique(s) do you use? (Tick all that apply)

- Ultracentrifuge wash
- Density gradient (please state method/manufacturer)
- Liquid chromatography (please state method/manufacturer)
- Other (please specify below)
- Comments

5. How do you characterise your isolated vesicles routinely? (Tick all that apply)

- Western blotting
- Direct flow cytometry (Please specify instrument)
- Flow cytometry after capture on beads (Please specify bead manufacturer and instrument)
- Single particle analysis, e.g., NTA, TRPS, etc. (please state method/manufacturer)
- Electron microscopy
- Atomic force microscopy
- Protein assay (please state method/manufacturer)
- Other (please specify below)

6. Which additional characterisations do you perform and how frequently?

- Comments

7. What are the downstream applications for your isolated vesicles? (Tick all that apply)

- Proteomics (please state methods)
- RNA analysis (please state methods)
- *In vivo* EV functional assay
- *In vitro* EV functional assay
- Other (please specify below)

8. From how many samples a month do you isolate EV?

- less than 10
- 11 to 50
- 51 to 100
- Over 100

9. Other comments

Table S2. Starting material (%)

Application	Conditioned cell culture media (serum added)	Conditioned cell culture media (serum-free)	Plasma	Serum	Urine	Cerebro spinal fluid	Milk	Other body fluid	Non-mammalian
All	52	56	47	22	14	8	5	13	2
RNA	50	50	52	27	11	9	6	13	3
Flow cytometry	54	59	63	22	17	10	7	15	1
***In vitro* function**	59	63	44	24	12	6	6	14	1
***In vivo* function**	57	64	48	39	11	9	4	14	0
Proteomics	48	52	55	25	18	17	7	15	3

Table S3. Primary isolation technique (%)

Application	Ultracentrifugation	Precipitation	Density gradient centrifugation	Filtration	Size exclusion chromatography	Magnetic bead capture	Combination of methods	Other
All	81	14	20	18	15	9	59	4
RNA	83	20	21	17	14	10	61	3
Flow cytometry	81	11	28	15	19	15	65	4
***In vitro* function**	87	12	20	21	16	10	59	3
***In vivo* function**	89	11	18	21	14	5	61	4
Proteomics	84	14	24	16	18	13	70	2

Table S4. Additional clean up by downstream application (%)

Application	Ultracentrifuge wash	Density gradient centrifugation	Liquid chromatography	Other
All	64	27	20	6
RNA	68	29	18	8
Flow cytometry	63	28	27	6
***In vitro* function**	67	30	21	4
***In vivo* function**	70	29	9	2
Proteomics	70	37	20	9

Table S5. Combinations of applications used (%)

	Primary method					
Secondary method	**All**	**RNA**	**Flow cytometry**	**Proteomics**	***In vitro* function**	***In vivo* function**
RNA	60	-	59	70	59	57
Flow Cytometry	41	41	-	38	46	39
Proteomics	47	56	44	-	48	54
Lipidomics	5	5	9	6	6	2
***In vitro* function**	72	71	77	73	-	96
***In vivo* function**	29	27	26	32	38	-
Other	8	3	5	4	4	7